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SOME PHYSICAL CHARACTERISTICS OF CORNING POROUS GLASS PACKING IN GEL PERMEATION CHROMATOGRAPHIC SEPARATIONS

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SUMMARY

The nature of the interactions between the main gel permeation chromatography (GPC) components, *viz.*, the solute, eluent and gel, was examined. Corning controlled porosity glass (CPG) was chosen as the column packing and the separation of low-molecular-mass organic molecules, polystyrene standards and narrow-molecular-mass-distribution poly-2-vinylpyridine samples was carried out. Pure eluents (methyl ethyl ketone and dimethylformamide) and mixed eluents (methyl ethyl ketone–ethanol, methyl ethyl ketone–acetone and dimethylformamide–methanol) were used. The results showed that the attractive physical forces between solutes and CPG also contribute to the mechanism of separation. These forces could be suppressed or avoided by changing the nature of the solvent.

INTRODUCTION

Gel permeation chromatography (GPC) as a method for the characterization of polymers continues to be of increasing importance in relation to the molecular mass distribution. It enables a continuous recording of polymer concentration as a function of elution volume to be made. The chromatographic patterns obtained can be converted by appropriate calibration into molecular mass or molecular mass distribution values. Both the calibration and the interpretation of chromatograms are limited, however, if the solutes display a preferential affinity for the mobile phase, the stationary phase or the gel. In addition, each solute may interact to a certain extent with the eluent or gel, depending on the nature of the solute and the eluent and the polarity of the gel.

Consequently, in such systems the size exclusion mechanism controlled by solute size is not the only mechanism of separation, and a second mechanism also exists[1–3]. Therefore, for exact GPC analysis it is necessary to know the nature of these non-size-exclusion effects.

A number of workers[4–10] have investigated solute–gel interactions in recent years but little information is available on these non-size-exclusion effects or on methods for their elimination or reduction. Yano and Janado[9] found that a homologous series of aliphatic *n*-alcohols can be separated by gel chromatography on a

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column of unsubstituted Sephadex G-10. The mechanism of separation was studied in detail.

This study was undertaken to elucidate the nature of the interactions that occur on the polar surface of controlled porosity glass (CPG) during permeation chromatography. First, the nature of the interactions between low-molecular-mass organic components with widely varying polarities and different affinities towards the polar CPG were investigated. Second, the possibility of suppressing the attractive physical forces between the solute and the gel was tested with poly-2-vinylpyridine (P2VP) as a polar polymer.

EXPERIMENTAL

Materials

All of the low-molecular-mass solutes were of analytical-reagent grade (E. Merck, Darmstadt, G.F.R.). Eluents were distilled immediately before use. Analytical-reagent grade dimethylformamide (DMF) was purified by distillation over calcium hydride under reduced pressure at 363°K.

A series of well defined polystyrene (PS) standards (Waters Assoc., Milford, MA, U.S.A., ArRo Labs., Joliet, IL, U.S.A. and Pressure Chem., Pittsburgh, PA, U.S.A.) with molecular masses in the range $1.8 \cdot 10^3$–$2.7 \cdot 10^6$ were used. P2VP samples were obtained by courtesy of the Centre de Recherches sur les Macromolecules (Strasbourg, France), with molecular masses in the range $6.0 \cdot 10^3$–$1.34 \cdot 10^5$.

Procedures

The Waters Assoc. GPC set-up, with a Model 6000 A solvent delivery system, Model U6K universal injector and R 401 differential refractometer was used.

Controlled porosity glasses manufactured by Corning (Corning, NY, U.S.A.) and distributed by Waters Assoc. with pore sizes of 75, 240 and 1250 Å were used as the materials for GPC separation. The CPGs were packed with vibration as dry material into stainless-steel columns of length 1218 mm and I.D. 6 mm. After packing the columns were pumped with eluents for 48 h at a flow-rate of 0.1 cm^3/min. All columns were operated at ambient temperature. The elution rate was 1 cm^3/min and the volumes injected were 0.015 cm^3 for the low-molecular-mass solutes and 0.5 cm^3 (0.2%) for the polymer solutions.

RESULTS AND DISCUSSION

The elution behaviour of low-molecular-mass solutes in methyl ethyl ketone (MEK) is shown in Fig. 1 as plots of logarithm of molar volume *versus* elution volume.

The same retention of all low-molecular-mass solutes is to be expected because the size of the solvated molecules is much smaller than the mean pore size in the gel so that all the molecules can penetrate deeply into the gel. However, non-polar and moderately polar components and aprotic polar low-molecular-mass solutes are eluted with smaller retentions than molecules that are very polar or proton donors (DMF, water, alcohols). The elution volumes of alcohols increase with decreasing alkyl chain length. Such large differences in elution volume of alcohols cannot, there-

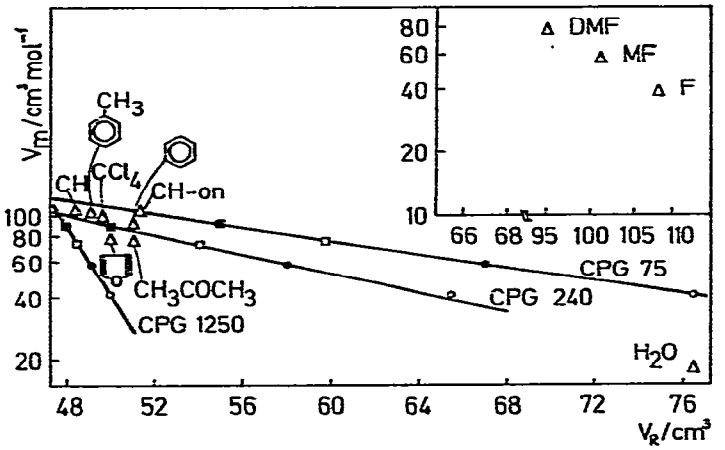

Fig. 1. Molar volume, V_m, *versus* retention volume, V_R, calibration graphs in the following columns: (1) CPG 75, (2) CPG 240 and (3) CPG 1250 in MEK at 293°K. ○, Methanol; ●, ethanol; □, *n*-propanol; ■, *n*-butanol; △, other low-molecular-mass solutes. Inset: elution behaviour of dimethylformamide (DMF), methylformamide (MF) and formamide (F) in CPG 240 column.

fore, reflect steric exclusion retention. The same order of elution of the alcohols was observed by other workers[9] in hydrophobic interaction chromatography.

The injection of each alcohol was repeated twenty times on the CPG columns. A change in retention towards lower elution volumes was observed (Fig. 2). Afterwards the column was flushed at a slightly elevated temperature (303°K) with the same eluent (MEK) for 24 h, applying the same flow-rate of 1 cm³/min. The previous values of the elution volumes were obtained and remained unmodified.

The question arises of what the driving force in the separation of low-molecular-mass solutes is. It is evident (Fig. 1) that components in these experiments are separated by a second mechanism resulting from gel–solute interactions. These attractive physical forces, considering the chemical composition, are dipole–dipole interactions or hydrogen bonds in the case of solutes which are proton donors.

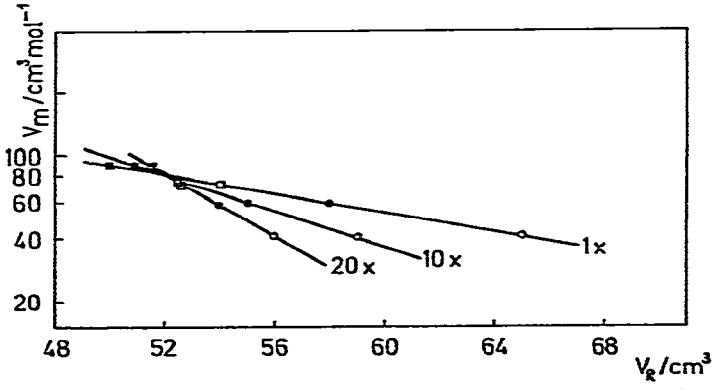

Fig. 2. Shift of the molar volume *versus* retention volume calibration graph for the CPG 240 column in MEK at 293°K by repeated injection (10 and 20 times) of (○) methanol, (● ●) ethanol, (□) *n*-propanol and (■) *n*-butanol.

The magnitude of the attractive physical forces depends on the surface area of the CPG. The larger the surface area for adsorption (for CPG 240 the surface area is 70.0 m^2/g and for CPG 1250 it is 10.6 m^2/g)[10], the greater are the differences in the elution volumes of alcohols (Fig. 1). It seems that some solutes remain irreversibly adsorbed on the CPG, owing to the strong bonding characteristics of the active sites on the surface of the CPG, and consequently the results are not reproducible (Fig. 2).

The above considerations are consistent with the data on solubility parameters. MEK as an eluent and alcohols as solutes differ in their polarities, as indicated by the solubility parameters in Table I. Thus, the highly polar CPG prefers to interact with more polar alcohols than with MEK.

In further investigations we tried to avoid or at least suppress the preferential interactions between CPG and low-molecular-mass organic solutes. A criterion for pure exclusion was the absence of any effect of the polarity of small organic solutes on the elution volume. On the basis of the elution of PS (M_w = 1800) in MEK and other eluents, the total permeation volume in the CPG 240 column was estimated to be about 48 cm^3, which should be the assumed elution volume of low-molecular-mass solutes for pure steric exclusion. The eluent is the only component that could be modified or changed. Therefore, DMF as a very polar single eluent and some mixed eluents were used. From the elution volumes of alcohols in Fig. 3 and considering the solubility parameters, we can conclude that the elution behaviour of solutes depends on the polarity of the eluent. The most important factor, however, is the type of interactions between the eluent, solute and gel. For example, the elution volumes of alcohols in DMF [$\delta^0 = 24.88 \cdot 10^3$ $(J/m^3)^{1/2}$] as a single very polar eluent are higher than in a less polar mixed eluent, MEK–ethanol [$\delta^0 = 20.38 \cdot 10^3$ $(J/m^3)^{1/2}$] (Fig. 3 and Table I). This is due to the presence of hydroxyl groups in ethanol and to the fact that DMF is an aprotic solvent. Ethanol, from the mixed eluent, is presumably adsorbed on the surface of CPG, and injected alcohols and the eluent are in com-

TABLE I[11]

SOLUBILITY PARAMETERS IN $(J/m^3)^{1/2} \cdot 10^{-3}$

Compound	δ^0	δ_d	δ_p	δ_h
Acetone	20.03	15.54	10.46	6.97
Methyl ethyl ketone	19.00	15.93	9.02	5.13
Methanol	29.27	15.21	12.30	22.35
Ethanol	25.49	15.85	8.82	19.48
Propanol	24.54	15.89	6.77	17.43
Butanol	23.17	16.01	5.74	15.79
Cyclohexane	16.80	16.80	0.00	0.00
Benzene	18.76	18.35	1.03	2.05
Dimethylformamide	24.88	17.46	13.74	11.28
Methyl ethyl ketone–18% ethanol	20.38*			
Methyl ethyl ketone–50% acetone	19.52*			
Dimethylformamide–30% methanol	25.93*			

* The total solubility parameter, δ^0, of mixed eluents was calculated according to the equation in ref. 12.

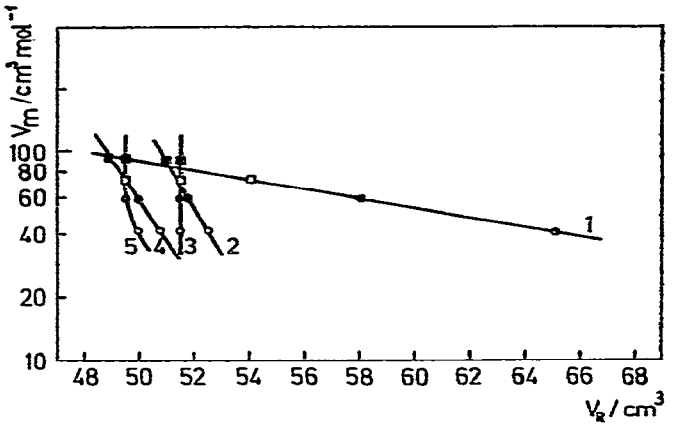

Fig. 3. Molar volume *versus* retention volume calibration graphs for the CPG 240 column in (1) MEK, (2) MEK–50% acetone, (3) DMF–30% methanol, (4) DMF and (5) MEK–18% ethanol for (○) methanol, (●) ethanol, (□) *n*-propanol and (■) *n*-butanol.

petition for the surface sites. Only more polar methanol can displace ethanol and other alcohols are therefore eluted at the same volume. Similarly, the elution volumes of alcohols in MEK–acetone [$\delta^0 = 19.52 \cdot 10^3$ (J/m^3)$^{1/2}$] are higher than the corresponding values in other mixed eluents, because both components are aprotic. Further, the retention of alcohols in DMF–30% MeOH [$\delta^0 = 25.93 \cdot 10^3$ (J/m^3)$^{1/2}$] is the same, which can also be explained by the solubility parameters of the solutes and the eluent.

The experiments described above were carried out on all the CPG columns, but only the results with column CPG 240 are reported here.

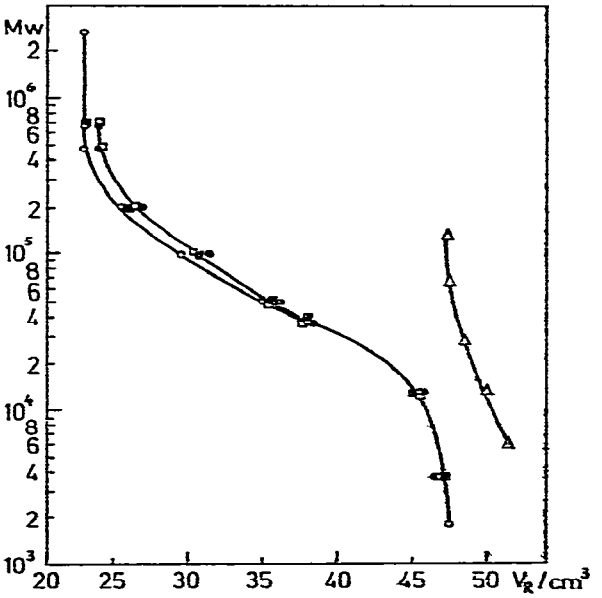

Fig. 4. Molecular mass *versus* retention volume calibration graphs for the CPG 240 column: ○, PS–MEK; ●, PS–MEK–18% ethanol; □, PS–MEK–50% acetone; ■, PS–DMF; △, P2VP–DMF–30% methanol.

In the second part of our work we investigated the influence of the polarity of the eluent on the GPC separation of macromolecular solutes, PS and P2VP, on CPG in the same eluents as before. The results on the CPG 240 column are presented in Fig. 4 as plots of logarithm of molecular mass *versus* elution volume.

All of the specific calibration graphs for PS as a moderately apolar polymer[2] in Fig. 4 are of sigmoid shape and located at similar elution volumes. They do not depend strongly on the solvent power of the eluent. CPG is a rigid glass and therefore the total interstitial volume and/or the total internal gel volume for the column does not depend on the type of eluent.

Different slopes might, however, be expected owing to the fact that the eluents used have different solvent powers for PS when separation is not dependent only on the solute size. As this phenomenon is not very pronounced, it might be concluded that PS–solvent interactions in the eluents used do not differ greatly or that in none of the systems do preferential interactions among the solute, eluent and gel appear to be very active.

As all of the solvents (eluents) used are more polar than PS, the latter cannot compete for the surface sites so that eluents are preferentially adsorbed on the polar CPG and steric exclusion is the predominent mechanism of PS separation. This conclusion is consistent with the results on the low-molecular-mass solutes in our investigation. Detailed discussion on the nature of a possible secondary mechanism cannot be carried out without a universal calibration plot and the Mark–Houwink constant, a, for the above-mentioned systems.

The next polymer used was P2VP. PS and P2VP have similar structures but the free electron pair on the nitrogen atom in P2VP makes it more polar than PS. P2VP is soluble in the same single and mixed eluents used above for PS separation. None of these eluents separates P2VP on CPG. P2VP completely failes to elute in MEK and DMF and gives a large tailing peak in MEK–18% ethanol. Such behaviour of P2VP indicates strong interactions, responsible for the adsorption of P2VP on CPG, which may be explained by the solvent power of the eluents (Mark–Houwink constants, a, solubility parameters, δ^0) and/or by the type of interactions among the main components in the GPC system. MEK is a poor solvent for P2VP [$a = 0.47$; $\delta^0_{MEK} = 19.0 \cdot 10^3$ $(J/m^3)^{1/2}$; $\delta^0_{P2VP} = 21.5 \cdot 10^3$ $(J/m^3)^{1/2}$] and P2VP will prefer the polar gel environment to the mobile phase. DMF [$a = 0.72$; $\delta^0 = 24.88 \cdot 10^3$ $(J/m^3)^{1/2}$] is better, but is still an aprotic solvent and not sufficiently strong to separate P2VP. Considering the previous results obtained with low-molecular-mass solutes, more polar protic solvents should be used.

The chromatograms of P2VP samples in Fig. 5 obtained in the mixed eluents DMF–10% methanol and DMF–20% methanol indicate progress in elution but adsorption still occurs, because all of the low-molecular-mass samples leave the column at the same place. Therefore, DMF–30% methanol was used as the mobile phase and solvent. The results of separation in this eluent are shown in Fig. 4 (right-hand curve). It is evident that the separation occurred at the end. Methanol interacts preferentially with CPG and probably deactivates the original sites of adsorption. When a sufficient concentration of methanol is used (30%), deactivation progresses and P2VP samples were less retained and finally separated according to a complex mechanism, still consisting of steric exclusion and adsorption.

An attempt to improve the separation of P2VP on CPG and the possibility of

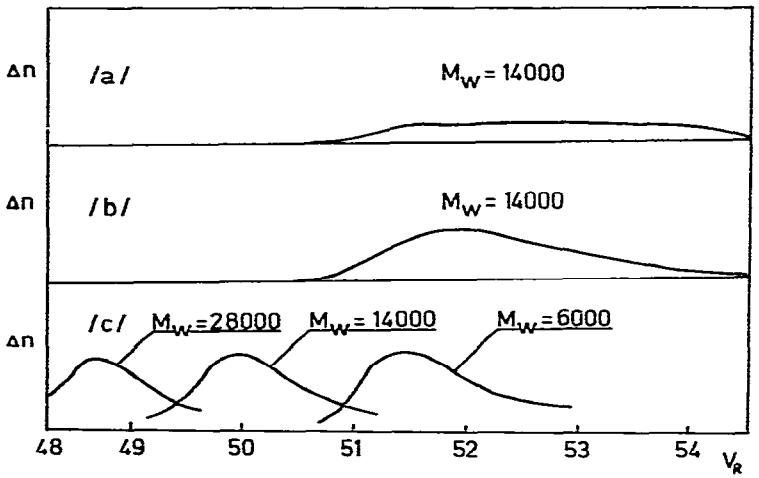

Fig. 5. Chromatograms obtained on the CPG 240 column in the systems (a) P2VP–DMF–10% methanol, (b) P2VP–DMF–20% methanol and (c) P2VP–DMF–30% methanol.

separating other polar polymers (polyacrylonitrile) will be the subject of further investigation.

CONCLUSION

CPG is a very polar column packing with strong proton-accepting characteristics. Low-molecular-mass organic solutes can therefore be separated according to their proton-donating characteristics.

The separation of protic components in aprotic eluents on CPG is not reproducible owing to the strong interactions, which may be irreversible under the given conditions.

Attractive physical forces between solutes and CPG, which contribute to the second mechanism of separation, can be suppressed or avoided using mixed, more polar eluents.

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